Amendments to the Claims:

1. (Currently amended) A method for purifying target molecules from a primer extension sequencing reaction using a purification device comprising the following steps:

(a) introducing the primer extension sequencing reaction mixture into a purification device comprising an electrophoretic medium containing immobilized capture probes, wherein the immobilized capture probes are selected from the group consisting of nucleic acids, modified nucleic acids and nucleic acid analogs;

(b) subjecting the electrophoretic medium of step (a) to an electric field resulting in the electrophoretic migration of one, or more, target molecules into at least one region of the electrophoretic medium containing immobilized capture probes, wherein the target molecules bind to the immobilized capture probes and non-target molecules continue to migrate under the influence of the electric field, thereby separating the target molecules from the non-target molecules of the primer extension sequencing reaction mixture;

(c) imposing conditions on the electrophoretic medium that dissociate the targets and their complementary capture probes;

(d) applying an electric field while maintaining the dissociating conditions within the electrophoretic medium, thereby causing the dissociated target molecules to exit the electrophoretic medium by electrophoretic migration; and

(e) collecting the purified target molecules that ~~which~~ have exited the electrophoretic medium.

2. (Original) The method of Claim 1, wherein the purification device is a microtiter plate.

3. (Original) The method of Claim 2, wherein the microtiter plate comprises multiple wells.

4. (Original) The method of Claim 3, wherein the number of wells contained within the microtiter plate is selected from the group consisting of: 6, 12, 48, 96 and 384.

5. (Currently amended) The method of Claim 1, wherein in step (c), a sufficient voltage is applied to release the target molecules from their ~~its~~ complementary capture probes, and wherein the released target molecules continue[~~s~~] electrophoretic migration under the influence of an electric field and exit[s] the electrophoretic medium, and wherein the purified, released target molecules ~~it~~ collect[~~s~~] in a collecting chamber.

6. (Currently amended) The method of Claim 5, wherein the polarity of the electric field is reversed, wherein the released target molecules ~~will~~ migrate back toward the test sample receptacle and ~~wherein it is~~ are subject to collection.

7. (Currently amended) The method of Claim 1, wherein the capture probes are ~~is a~~ nucleic acid molecules.

8. (Currently amended) The method of Claim 7, wherein the capture probes are ~~is~~ complementary to the primer extension sequencing product.

9. (Currently amended) The method of Claim 8, wherein the capture probes are ~~is~~ from about 20 to about 2000 nucleotides in length.

10. (Currently amended) A method for purifying multiple sets of primer extension sequencing reaction products which are formed by synthesizing multiple sets of primer extension sequencing reaction products comprising the following steps:

(a) introducing the multiple sets of primer extension sequencing reaction products into a purification device comprising at least two cartridges, wherein each cartridge comprises an electrophoretic medium containing at least one unique set of immobilized capture probes, and wherein the immobilized capture probes are selected from the group consisting of nucleic acids, modified nucleic acids and nucleic acid analogs;

(b) subjecting the electrophoretic media of step (a) to an electric field resulting in the electrophoretic migration of one, or more, sets of primer extension sequencing reaction products into the cartridges of step (a), wherein target molecules in each set of primer extension sequencing reaction products bind to a substantially complementary ~~complimentary~~ set of immobilized capture probes and non-target molecules continue to migrate under the influence of the electric field, thereby separating the target molecules from the non-target molecules in each set of the primer extension sequencing reaction products;

(c) imposing conditions on the electrophoretic media ~~medium~~ that dissociate the target molecules and their complementary capture probes;

(d) applying an electric field while maintaining the dissociating conditions within the electrophoretic media ~~medium~~, thereby causing the dissociated target molecules to exit the electrophoretic media ~~medium~~ by electrophoretic migration; and

(e) collecting each set of purified primer extension sequencing reaction target molecules that ~~which~~ exit the electrophoretic media ~~medium~~.

11. (Original) The method of Claim 10, wherein the purification device is a microtiter plate.

12. (Original) The method of Claim 11, wherein the microtiter plate comprises multiple wells.

13. (Currently amended) The method of Claim 12, wherein the number of wells contained within the microtiter plate is selected from the group consisting of: 6, 12, 48, 96 and 384.

14. (Currently amended) The method of Claim 10, wherein in step (c), a sufficient voltage is applied to release the target molecules from their ~~its~~ complementary capture probes, and wherein the released target molecules continue[s] electrophoretic migration under the influence of an electric field and exit[s] the electrophoretic media ~~medium~~, and wherein the purified, released target molecules ~~it~~ collect[s] in a collecting chamber.

15. (Currently amended) The method of Claim 14, wherein the polarity of the electric field is reversed, wherein the released target molecules ~~will~~ migrate back toward the test sample receptacle and ~~wherein it is~~ are subject to collection.

16. (Currently amended) The method of Claim 10, wherein the capture probes are ~~is a~~ nucleic acid molecules.

17. (Currently amended) The method of Claim 16 ~~17~~, wherein the capture probes are ~~is~~ complementary to the primer extension sequencing products.

18. (Currently amended) The method of Claim 17, wherein the capture probes are ~~is~~ from about 20 to about 2000 nucleotides in length.

19. (Currently amended) A method for purifying target molecules from a primer extension sequencing reaction using a purification device comprising the following steps:

(a) introducing the primer extension sequencing reaction mixture into a purification device comprising an electrophoretic medium containing capture probes modified with a 5'-acrylamide moiety and selected from the group consisting of nucleic acids, modified nucleic acids and nucleic acid analogs, wherein the capture probes are immobilized in the electrophoretic medium by a covalent bond between the 5'-acrylamide moiety and the electrophoretic medium;

(b) subjecting the electrophoretic medium of step (a) to an electric field resulting in the electrophoretic migration of one, or more, target molecules into at least one region of the electrophoretic medium containing immobilized capture probes, wherein the target

molecules bind to the immobilized capture probes and non-target molecules continue to migrate under the influence of the electric field, thereby separating the target molecules from the non-target molecules of the primer extension sequencing reaction mixture;

(c) imposing conditions on the electrophoretic medium that dissociate the targets and their complementary capture probes;

(d) applying an electric field while maintaining the dissociating conditions within the electrophoretic medium, thereby causing the dissociated target molecules to exit the electrophoretic medium by electrophoretic migration; and

(e) collecting the purified target molecules that ~~which~~ have exited the electrophoretic medium.

20. (Original) The method of Claim 19, wherein the capture probes are immobilized in the electrophoretic medium by copolymerizing the 5'-acrylamide moiety with the electrophoretic medium.

REMARKS

Claims 1-20 are pending in this application. All claims stand rejected under 35 U.S.C. § 103(a) as obvious over Japanese Unexamined Patent Appln. No. H3[1991]-47097 (Jiro *et al.*; published February 28, 1991) [hereinafter "Jiro"] in combination with at least one secondary reference. In response, Claims 1, 5-10, and 14-19 have been amended. Claims 1, 5, 10, 14, and 19 have been amended to recite that the target molecules are purified upon exit from the electrophoretic medium. Support for this amendment is found in the preamble of original Claims 1, 10, and 19 and throughout the specification, for example at page 5, lines 17-28. Claims 1, 5-10 and 14-19 have also been amended to correct certain typographical errors. As such, none of these amendments introduces new matter. Favorable reconsideration and allowance of all pending claims in view of these amendments and the following remarks are respectfully requested.

To establish a case of *prima facie* obviousness, the cited references must teach or suggest every claim limitation, and the prior art must provide a motivation to combine the cited references and a reasonable expectation of success (MPEP § 2142).

As Jiro is cited as the primary reference in all current rejections, Applicants will address the relevance of this reference first, and then discuss its combination with the secondary references as applied to individual claims.

Primary Reference:
Japanese Unexamined Patent Appln. No. H3[1991]-47097 (Jiro *et al.*)

Jiro teaches a method of hybridizing a nucleic acid sample with a nucleic acid probe that is fixed in an electrophoretic carrier (page 8, lines 10-15 & Claim 1). The nucleic acid sample migrates, by electrophoresis, through the

electrophoretic carrier (page 8, lines 13-15). As the sample migrates, those nucleic acids that are sufficiently complementary to the fixed nucleic acid probe are bound thereto, while non-complementary nucleic acids continue to migrate through the carrier (page 12, lines 17-21). Jiro teaches only that this hybridization method may be used to detect genetic mutations in a nucleic acid sample (page 6, lines 6-9; page 8, line 21 – page 9, line 25; & claims 2-7).

In contrast, the present claimed invention pertains to methods of purifying a target molecule contained within a test sample (*see, e.g.*, page, 2, lines 21-22). Claim 1 recites: "A method for purifying target molecules from a primer extension sequencing reaction using a purification device...." Specifically, independent Claims 1, 10, and 19 are directed to a method for purifying target molecules from a primer extension sequencing reaction (or multiple sets thereof, as recited in Claim 10) using a purification device comprising an electrophoretic medium containing immobilized capture probes. As amended, independent Claims 1, 10, and 19 emphasize the intended purposes of the claimed method by specifying that purified target molecules are collected upon exiting the electrophoretic medium. A close reading of Jiro reveals that it is, in fact, irrelevant to the patentability of these claimed purification methods; Jiro contains no teaching or suggestion that the hybridization method disclosed therein may be used to purify target molecules contained within a test sample. Consequently, Applicants submit Jiro may not be relied upon as "expressly teaching a method for purifying nucleic acid target molecules from a reaction using a purification device" (Office Action dated February 19, 2003, page 3, lines 1-2 [hereinafter "Office Action"]). Moreover, Jiro provides no motivation for its combination with any other reference to attain the claimed methods, let alone a reasonable expectation of success.

As a further point of distinction, Applicants note that their claimed purification method utilizes "target molecules from a primer extension sequencing reaction" (*see, e.g.*, Claim 1). While the method taught by Jiro employs nucleic acid molecules, it contains neither an explicit nor implicit indication that those nucleic acid molecules may be derived from a primer extension sequencing reaction.

Thus, in view of the points of distinction provided above, Applicants submit that Jiro fails to teach or suggest a "method for purifying target molecules from a primer extension sequencing reaction," as recited in the pending claims, and, thereby, fails as the primary reference in the *prima facie* case of obviousness presented by the Examiner. Applicants further submit that, as discussed below, none of the cited secondary references remedy the deficiencies of Jiro. Consequently, the obviousness rejections cannot be sustained.

Rejection of Claims 1, 5, 7, and 19 Under 35 U.S.C. §103(a)

Claims 1, 5, 7, and 19 stand rejected as obvious over Jiro in view of Gelfi *et al.* (1996) "Temperature-Programmed Capillary Electrophoresis for Detection of DNA Point Mutations" *BioTechniques* 21(5):926-32 [hereinafter "Gelfi"]. Applicants respectfully traverse this rejection.

The rejected claims are directed to a method for purifying target molecules from a primer extension sequencing reaction using a purification device comprising an electrophoretic medium containing immobilized nucleic acid capture probes (Claims 1, 7, & 19). Following electrophoresis, the target molecules may be dissociated from the capture probes to which they are hybridized by applying a sufficient voltage to the electrophoretic medium (Claim

5). These claims are nonobvious over the applied art, as discussed more fully below.

Gelfi teaches that point mutations in DNA heteroduplexes may be identified using capillary electrophoresis (pages 927-28). Specifically, Gelfi teaches an electrophoretic technique that exploits the differential denaturation temperatures of various DNA heteroduplexes, *i.e.* wild type – wild type, mutant – mutant, and wild type – mutant (page 930). The temperature gradient in the electrophoretic medium is generated using voltage ramps: voltage increases within the fused-silica capillaries produce predictable temperature increases (pages 927-28).

The Examiner has suggested that it would have been *prima facie* obvious to one skilled in the art at the time the invention was made to combine the method of Jiro with the teachings of Gelfi in order to effect release of the hybridized nucleic acid sample from the electrophoretic medium (Office Action, page 4, lines 5-9). However, as discussed above, Jiro is directed to a method of hybridizing nucleic acids to detect genetic mutations and does not teach or suggest the claimed method of purifying target molecules from a primer extension sequencing reaction. Gelfi is similarly directed to a method of detecting genetic mutations and also lacks any teaching of a purification method. Gelfi, therefore, cannot remedy the shortcomings of Jiro. Thus, the teaching in Gelfi that voltage increases may be used to effect temperature increases in electrophoretic media is alone insufficient to sustain the obviousness rejection of Claims 1, 5, 7, and 19. Accordingly, Applicants respectfully request that this rejection be reconsidered and withdrawn.

Rejection of Claims 2-4, 10-14, and 16 Under 35 U.S.C. §103(a)

Claims 2-4, 10-14, and 16 stand rejected as obvious over Jiro in view of Gelfi and further in view of U.S. Patent No. 5,482,836 (Cantor *et al.*) [hereinafter "Cantor"]. Applicants respectfully traverse this rejection.

The rejected claims are directed to a method for purifying target molecules from a primer extension sequencing reaction using a purification device comprising an electrophoretic medium containing immobilized nucleic acid capture probes, wherein said device is a microtiter plate (Claims 2 & 11), which may include multiple wells (Claims 3-4 & 12-13). The device may include multiple sets of unique capture probes (Claims 10, 14, & 16). As provided below, the combination of Jiro and Cantor does not render obvious Applicants' claimed methods.

Cantor teaches a method of isolating double stranded DNA (dsDNA) using triple helix formation (col. 3, lines 1-3). The mixture containing the dsDNA is contacted with an oligonucleotide that is designed to form a triple helix with the target dsDNA (col., 5, lines 61-66). The oligonucleotide is coupled to a first recognition molecule of a specific molecular recognition system (col. 5, lines 61-65). The triple helices are then exposed to a solid carrier that includes a second recognition molecule from the same molecular recognition system (col. 6, lines 12-18). Thus, the triple helices containing the target dsDNA are isolated from the mixture as the recognition molecules bind each other. The dsDNA is further isolated by treating the solid phase-triple helix conjugates with a reagent (*e.g.* a basic buffer) that breaks the bonds between the oligonucleotide and the dsDNA (col. 6, lines 20-28). Molecular recognition systems suitable for use in this method include antigen/antibody, avidin/biotin, streptavidin/biotin, protein

A/Ig, and lectin/carbohydrate systems (col. 7, lines 13-16). Cantor also teaches that:

> another interesting variation of this invention would be multiplexing or using two or more different traps so as to isolate two or more different targets from a single mixture. Combined use of other efficient labeling and molecular recognition capturing systems (*e.g.*, digoxigenin and antidigoxigenin antibody) with the biotin-streptavidin system would enable one to recover multiple target DNAs

(col. 12, lines 46-52).

The Examiner has suggested that it would have been *prima facie* obvious to modify the assay taught by Jiro and Gelfi by multiplexing with microtiter plates or using two or more different traps so as to isolate two or more different targets from a single mixture (Office Action, page 5, lines 7-12). However, as discussed above, the combination of Jiro and Gelfi fails to teach or suggest the claimed method of purifying target molecules from a primer extension sequencing reaction. Although Cantor is directed to a method of purifying DNA, its teachings are exclusively directed to purification of dsDNA. In fact, Cantor's methodology cannot be used with target molecules from a primer extension sequencing reaction (*i.e.* single stranded DNA (ssDNA)) because such molecules will not form triple helices when combined with an oligonucleotide. Thus, the combination of Jiro, Gelfi, and Cantor does not suggest Applicants' claimed invention.

More fundamentally, one skilled in the art would lack any motivation to combine the teachings of Cantor with those of Jiro and Gelfi. While Jiro and Gelfi disclose methods utilizing the hybridization characteristics of nucleic acid molecules (albeit methods irrelevant to Applicants' claimed purification

method), Cantor's methodology relies upon the binding characteristics of "molecular recognition systems," such as "antigen/antibody, avidin/biotin, a streptavidin/biotin, a protein A/Ig, and a lectin/carbohydrate system" (col. 7, lines 13-16). Cantor is entirely devoid of any suggestion that the binding characteristics of nucleic acid molecules themselves may be used to purify target molecules. Thus, the principles of operation underlying the methods of the references are incompatible, and they may not properly be combined to support an obviousness rejection (*See* MPEP §2143.01). Applicants respectfully request, therefore, that the obviousness rejection of Claims 2-4, 10-14, and 16 be reconsidered and withdrawn.

Rejection of Claims 8-9 and 17-18 Under 35 U.S.C. §103(a)

Claims 8-9 and 17-18 stand rejected as obvious over Jiro in view of Gelfi further in view of Cantor and further in view of U.S. Patent No. 4,683,202 (Mullis) [hereinafter "Mullis"]. Applicants respectfully traverse this obviousness rejection.

The rejected claims are directed to a method for purifying target molecules from a primer extension sequencing reaction using a purification device comprising an electrophoretic medium containing immobilized nucleic acid capture probes, wherein the capture probes are complementary to the primer extension sequencing product (Claims 8 & 17) and are between about 20 and about 2000 nucleotides in length (Claims 9 & 18). None of the references, either alone or in combination, teach or suggests Applicants' claimed invention with any reasonable expectation of success.

Mullis teaches a process for amplifying a desired nucleic acid sequence. Complementary oligonucleotide primers are hybridized to the ends of the

desired sequence, which serves as an initial template for the synthesis of complementary extension sequences from the primers (col. 6, lines 7-17). These extension sequences may, in turn, serve as templates for the synthesis of additional extension sequences (col. 8, lines 15-30 & col. 10, line 24 – col. 11, line 50). The process may be repeated as needed to obtain the necessary quantities of the desired nucleic acid sequence (col. 8, lines 31-36).

The Examiner suggests that one skilled in the art would have been motivated to incorporate Mullis' teaching of primer extension products into the method taught by Jiro, Gelfi, and Cantor in order to achieve the advantage of "producing nucleic acid sequences which are known to exist but are not completely specified" (Office Action, page 6, lines 7-20). Applicants respectfully disagree with this statement. As discussed above, the combination of Jiro, Gelfi, and Cantor fails to teach or suggest the claimed method of purifying target molecules from a primer extension sequencing reaction using a purification device comprising an electrophoretic medium containing immobilized nucleic acid capture probes. Nor does Mullis suggest that the products of a primer extension sequencing reaction may be incorporated into methods of purifying target molecules using electrophoretic media. The isolated teaching of primer extension sequencing products in Mullis is incapable of rendering the invention of Claims 8-9 and 17-18 obvious. Accordingly, Applicants respectfully request that this rejection be reconsidered and withdrawn.

Rejection of Claims 6 and 15 Under 35 U.S.C. §103(a)

Claims 6 and 15 stand rejected as obvious over Jiro in view of Gelfi and further in view of U.S. Patent No. 4,830,726 (Stamato *et al.*) [hereinafter "Stamato"]. Applicants respectfully traverse this rejection.

The rejected claims are directed to a method for purifying target molecules from a primer extension sequencing reaction using a purification device comprising an electrophoretic medium containing immobilized nucleic acid capture probes, wherein, following electrophoresis, the target molecules are dissociated from the capture probes to which they are hybridized by applying a sufficient voltage to the electrophoretic medium and the electric field is reversed such that the target molecules migrate back toward the test receptacle from which they originated for collection. As asserted below, Claims 6 and 15 are nonobvious over the combination of Jiro, Gelfi, and Stamato.

Stamato teaches a method of separating DNA molecules by gel electrophoresis in which alternating pulses of forward and reverse electric fields are applied to the electrophoretic medium (col. 3, lines 47-59). The DNA molecules migrate in the direction of the forward field because the product of the low potential voltage and longer pulse time interval for the forward field is greater than the product of the high potential voltage and shorter pulse time interval for the reverse field (col. 3, line 59 – col. 4, line 7). The migration distance of the DNA molecules in the forward direction is a function of their size (col. 4, lines 24-42).

The Examiner suggests that one skilled in the art would have combined the teachings of Stamato with the method taught by Jiro and Gelfi in order to provide easy removal and collection of the target molecules from the electrophoretic medium following their dissociation from the immobilized capture probes (Office Action, page 7, line 12 – page 8, line 10). Specifically, the Examiner points to the statement in Stamato that "one of skill in the art will acknowledge the applicability of this method to DNA from a variety of sources, other compositions appropriate for electrophoretic separation, and for a variety

of known uses of an electrophoretic method" (Office Action, page 8, lines 3-6 (quoting Stamato at col. 7, lines 27-32)). However, as discussed above, the combination of Jiro and Gelfi fails to teach or suggest the claimed method of purifying target molecules from a primer extension sequencing reaction using a purification device comprising an electrophoretic medium containing immobilized nucleic acid capture probes. Although Stamato is directed to an electrophoretic method of separating DNA molecules, it nowhere suggests the use of immobilized capture probes to purify target molecules from a primer extension sequencing reaction.

Moreover, Stamato teaches the use of alternating pulses of forward and reverse electric fields to separate DNA molecules. The use of a single reverse electric field to return target molecules to their point of origination after they have already been separated from the non-target components of the sample via hybridization with immobilized capture probes is neither taught nor suggested by Stamato. Stamato's broad statement that the alternating electric pulse method may be applied to other electrophoretic methods is, alone, too vague to be interpreted as suggesting the modification of the cited references so as to arrive at Applicants' claimed method of purification. The Examiner's conclusion that such a modification was obvious represents nothing more than impermissible hindsight reconstruction of Applicants' invention (*see, e.g.*, MPEP § 2145(X)(A)). Applicants respectfully request, therefore, that the obviousness rejection of Claims 6 and 15 be reconsidered and withdrawn.

Rejection of Claim 20 Under 35 U.S.C. §103(a)

Claim 20 stands rejected as obvious over Jiro in view of Gelfi and further in view of U.S. Patent No. 5,478,893 (Ghosh *et al.*) [hereinafter "Ghosh"]. Applicants respectfully traverse this rejection.

The rejected claim is directed to a method for purifying target molecules from a primer extension sequencing reaction using a purification device comprising an electrophoretic medium containing immobilized nucleic acid capture probes, wherein each capture probe is modified with a 5'-acrylamide moiety and immobilized via copolymerization of this moiety with the electrophoretic medium.

Ghosh teaches a method for covalently attaching oligonucleotides to solid supports (col. 7, lines 28-30). Specifically, Ghosh teaches oligonucleotides linked at their 5' end to polyacrylamide supports via a thioether covalent bond (col. 7, lines 30-32). The thioether bond is formed by reacting a nucleophilic thiol group linked to the 5' end of the oligonucleotide and a thiolphilic reactive carbon center group linked to the polyacrylamide support, or vice versa (col. 7, lines 32-38). Once attached, the oligonucleotides may be used in direct capture experiments (col. 27, lines 49-61).

The Examiner suggests that it would have been *prima facie* obvious to incorporate the covalently-attached oligonucleotides taught by Ghosh into the method taught by Jiro and Gelfi because Ghosh suggests the covalently-attached oligonucleotides may be used with nucleic acids in direct capture experiments (Office Action, page 9, lines 4-16). However, as discussed above, the combination of Jiro and Gelfi fails to teach or suggest the claimed method of purifying target molecules from a primer extension sequencing reaction using a

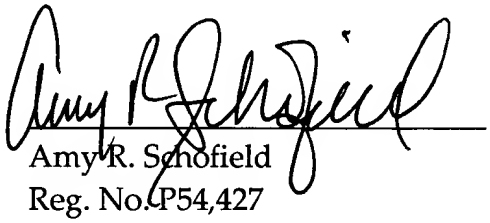
purification device comprising an electrophoretic medium containing immobilized nucleic acid capture probes. Nor does Ghosh suggest such a method. Although Ghosh does teach that oligonucleotides may be covalently attached to polyacrylamide gels, Ghosh does not teach or suggest that the oligonucleotides may be modified to contain a 5'-acrylamide moiety or may be attached to the electrophoretic medium in the manner claimed by Applicants: Claim 19 (the independent claim from which rejected Claim 20 depends) recites the use of "capture probes modified with a 5'-acrylamide moiety," and Claim 20 requires that the "capture probes are immobilized in the electrophoretic medium by copolymerizing the 5'-acrylamide moiety with the electrophoretic medium." Thus, Ghosh's teaching of oligonucleotides covalently attached to a polyacrylamide support via a thioether bond is irrelevant to the invention of Claim 20. The cited prior art combination, therefore, does not render the claimed method of purification obvious. Accordingly, Applicants respectfully request that this obviousness rejection of Claim 20 be reconsidered and withdrawn.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants submit the present claims are in condition for allowance. A timely Notice of Allowance is, therefore, respectfully requested. In order to expedite prosecution of this application, the Examiner is encouraged to contact the undersigned by telephone should any issues remain outstanding.

Applicants hereby petition for a two-month extension of time pursuant to 37 C.F.R. § 1.136 to respond to the Office Action mailed on February 19, 2003. Please deduct the corresponding $410.00 fee specified in 37 C.F.R. § 1.17(a)(2) from our Deposit Account No. 08-0219. No other fees are believed to be due in connection with this application. However, please charge any payments due or credit any overpayments to our Deposit Account No. 08-0219.

Respectfully submitted,
HALE AND DORR LLP



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